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Cytotoxicity test sTNF and scTNFs with neutralizing antibodies [AB]

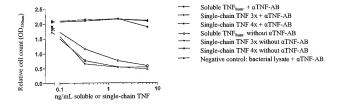


Figure 1

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Cytotoxicity test sTNF and scTNFs on MF-TNFR2

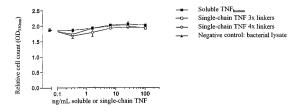
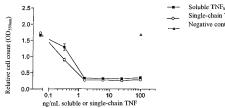


Figure 2

3/41

Cytotoxicity test sTNF and scTNFs on MF-TNFR2 + 80M2



Soluble TNF_{buman} + 80M2-AB
 Single-chain TNF 3x + 80M2-AB
 Negative control: bacterial lysate + 80M2-AB

Figure 3

4/41

Stability test with MF - freshly titrated

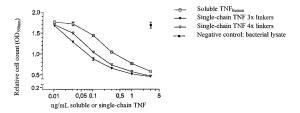


Figure 4

5/41

Stability test with MF - incubated for 8 days

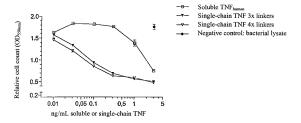


Figure 5

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Stability test with MF - incubated for 14 days

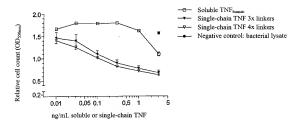


Figure 6

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Stability test with Kym1 - freshly titrated

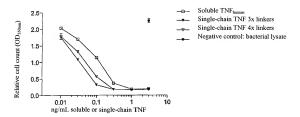


Figure 7

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Stability test with Kym1 - incubated for 16 days

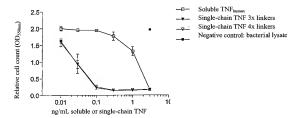


Figure 8

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Stability test with Kym1 - incubated for 22 days

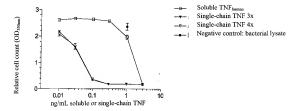


Figure 9

10/41

Stability test with Kym1 - titrated from stock solution

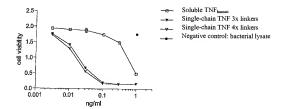


Figure 10

11/41

Stability test with human serum Serum stability - freshly titrated

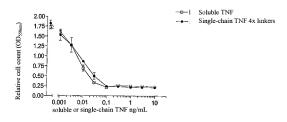


Figure 11

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Stability test with human serum Serum stability – 8 days

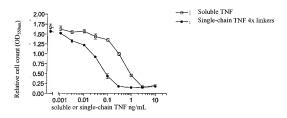


Figure 12

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Silver gel

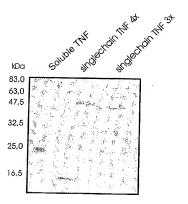


Figure 13

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Western Blot

Without β-mercaptoethanol soluble single-chain TNF soluble single-chain TNF

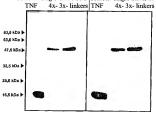


Figure 14

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IkB degradation assay

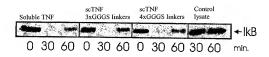


Figure 15

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JNK assay



Figure 16

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Electrophoretic Mobility Shift Assay (EMSA)

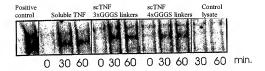


Figure 17

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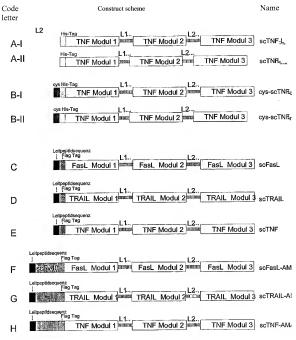


Figure 18

Key: Leitpeptidsequenz = leader peptide sequence Modul = module

19/41 Figure 19

ATG AGA GGA TCG CAT CAC CAT CAC CAT CAC GGA TCA GCG TCG TCT 48

Nucleic acid sequence and corresponding amino acid sequence of scTNF-L $_{\rm short}$ Construct A-II

1	M	G AG	A GG		G CA	r CA	H	r CA	C CA	T CA	G GG	A TC	A GC	F TC	S TCT S	45 15
46 16	TC:	r TC	T CG	T AC	C CC	G TC	GA(C AA	A CC	G GT	F GC!	E CAC	GT.	r gTi	GCA A	90 30
91 31	AA(C CC	G CA Q	G GC	GA/ E	A GG	CA2 Q	A CTO	G CAA	A TGC	CTC L	AAC N	CGT R	CGT R	GCT A	135 45
136 46	AAC N	GC: A	r cr L	G CTC	GCT A	AAC N	GGT G	GT'I	GA/ E	A CTC	G CGT	GAC D	: AAC N	CAG Q	CTG L	180 60
181 61	GTI V	GT:	P CC	TCT S	GAA E	G G	L L	TAC Y	CTC L	ATC I	TAC Y	TCC S	CAG Q	GTI V	CTG L	225 75
226 76	TTC	AA! K	G G	Q Q	GGC G	TGC	P	TCC S	ACC T	CAC H	GTI V	L	CTG L	ACC T	CAC H	270 90
271 91	ACC T	ATO	S	r CGT R	ATC	GCT	GTI V	TCC	TAC	CAG Q	ACC T	AAA K	GTA V	AAC N	CTG L	315 105
316 106	CTG L	TCT	GC#	ATC	AAA K	TCT	CCG	TGC	CAG	CGT R	GAA E	ACC	CCG	GAA E	GGT G	360 120
361 121	GCT A	GAA E	GCI A	AAA K	CCG	TGG W	TAC	GAA E	CCG	ATC	TAC	CTG	GGT G	GGC	GTT V	405 135
406 136	TTT	CAA Q	CTG L	GAG E	AAA K	GGT G	GAC D	CGT	CTG L	TCT	GCA A	GAA E	ATT I	AAC N	CGT R	450 150
451 151	CCG	GAC D	TAC	CTG L	GAC D	TTC F	GCA A	GAA E	TCT S	GGT G	CAG Q	GTT V	TAC Y	TTC F	GGT G	495 165
496 166	ATC I	ATC I	GCT A	CTG L	GGT G	GGC G	GGT G	TCT	GGT G	GGC G	GGT G	TCT S	GGT G	GGC G	GGA G	540 180
541 181	TCC S	TCT S	TCT	CGT R	ACC T	CCG	TCT	GAC D	AAA K	CCG P	GTT V	GCT A	CAC H	GTT V	GTT V	585 195
586 196	GCA A	AAC N	CCG P	CAG Q	GCT A	gaa E	GGT G	CAA Q	CTG L	CAA Q	TGG W	CTG L	AAC N	CGT R	CGT R	630 210
631 211	GCT A	AAC N	GCT A	CTG L	CTG L	GCT A	AAC N	GGT G	GTT V	gaa e	CTG L	CGT R	GAC D	AAC N	CAG Q	675 225
676 2 26	CTG L	GTT V	GTT V	P	TCT S	GAA E	GGC G	CTG L	TAC Y	CTG L	ATC I	TAC Y	TCC S	CAG Q	GTT V	720 240
721 241	CTG L	TTC F	AAA K	GGC G	CAG Q	GGC G	TGC C	CCG P	TCC	ACC T	CAC H	V GTT	CTG L	CTG L	ACC T	765 255
766 256	CAC :	ACC T	ATC I	TCT	CGT .	ATC I	GCT A	GTT V	TCC S	TAC Y	CAG Q	ACC T	aaa K	GTA V	AAC N	810 270
311 271	CTG (L L	TCT S	GCA .	ATC .	AAA '	TCT S	CCG P	TGC C	CAG Q	CGT R	GAA E	ACC T	CCG P	GAA E	855 285

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								20	41							
Continuation	Continuation of Figure 19															
856 286	GGT G	GCT A	GAA E	GCT A	K	CCG	TGG W	TAC	GAA E	CCG P	ATC I	TAC Y	CTG L	GGT G	GGC G	900 300
901 301	GTT V	TTT	CAA Q	CTG L	GAG E	AAA K	GGT	GAC D	CGT R		TCT	GCA A	GAA E	ATT I	AAC N	945 315
946 316	CGT R	CCG	GAC D	TAC	CTG L	GAC D	TTC F	GCA A	GAA E	TCT S	GGT G	CAG Q	GTT V	TAC Y	TTC F	990 330
991	GGT	ATC	ATC	GCT	CTG	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	1035
331	G		I	A	L	G	G	G	S	G	G	G	S	G	G	345
1036	GGA	TCC	TCT	TCT	CGT	ACC	CCG	TCT	GAC	AAA	CCG	GTT	GCT	CAC	GTT	1080
346	G		S	S	R	T	P	S	D	K	P	V	A	H	V	360
1081	GTT	GCA	AAC	CCG	CAG	gcT	gaa	GGT	CAA	CTG	CAA	TGG	CTG	AAC	CGT	1125
361	V	A	N	P	Q	A	E	G	Q	L	Q	W	L	N	R	375
1126	CGT	GCT	AAC	GCT	CTG	CTG	GCT	AAC	GGT	GTT	GAA	CTG	CGT	GAC	AAC	1170
376	R	A	N	A	L	L	A	N	G	V	E	L	R	D	N	390
1171	CAG	CTG	GTT	GTT	CCG	TCT	GAA	GGC	CTG	TAC	CTG	ATC	TAC	TCC	CAG	1215
391	Q	L	V	V	P	S	E	G	L	Y	L	I	Y	S	Q	405
1216	GTT	CTG	TTC	AAA	GGC	CAG	GGC	TGC	CCG	TCC		CAC	GTT	CTG	CTG	1260
406	V	L	F	K	G	Q	G	C	P	S		H	V	L	L	420
1261	ACC	CAC	ACC	ATC	TCT	CGT	ATC	GCT	GTT	TCC	TAC	CAG	ACC	AAA	GTA	1305
421	T	H	T	I	S	R	I	A	V	S	Y	Q	T	K	V	435
1306	AAC	CTG	CTG	TCT	GCA	ATC	AAA	TCT	CCG	TGC	CAG	CGT	GAA	ACC	CCG	1350
436	N	L	L	S	A	I	K	S	P	C	Q	R	E	T	P	450
1351	GAA	GGT	GCT	GAA	GCT	AAA	CCG	TGG	TAC	gaa	CCG	ATC	TAC	CTG	GGT	1395
451	E	G	A	E	A	K	P	W	Y	E	P	I	Y	L	G	465
1396	GGC	GTT	TTT	CAA	CTG	GAG	AAA	GGT	GAC	CGT	CTG	TCT	GCA	gaa	ATT	1440
466	G	V	F	Q	L	E	K	G	D	R	L	S	A	E	I	480
1441	AAC	CGT	CCG	GAC	TAC	CTG	GAC	TTC	GCA	gaa	TCT	GGT	CAG	GTT	TAC	1485
481	N	R	P	D	Y	L	D	F	A	E	S	G	Q	V	Y	495
1486 496	TTC F	GGT G	ATC I	ATC I	GCT A	CTG L	TGA *									1506 501

21/41 Figure 20

Nucleic acid sequence and corresponding amino acid sequence of cys-scTNF-L_{short} Construct B-II

1	AT M	G GG2	GAG E	CTC L	ATC I	GAA E	GGT G	CGC R	TGC	GCC A	GGT	GGA G	TCI	GGT G	CAT H	45 15
46 16	CA:	CAT H	CAC H	CAT	CAC H	GGC G	TC#	GAC D	GGA G	GCG A	TCG	TCT	TCT	TCT	CGT R	90 30
91 31	ACC T	C CCG	TCT	GAC D	AAA K	CCG	GTI V	GCT A	CAC H	GTT V	GTT V	GCA A	AAC N	CCG	CAG Q	135 45
136 46	GCT A	GAA E	GGT	CAA Q	CTG L	CAA Q	TGG	CTG L	AAC N	CGT R	CGT R	GCT A	AAC N	GCT A	CTG L	180 60
181 61	CTG	GCT A	AAC N	GGT G	GTT V	GAA E	CTG L	CGT	GAC D	AAC N	CAG Q	CTG L	GTT V	GTT V	CCG P	225 75
226	TCI	GAA	GGC	CTG	TAC	CTG	ATC	TAC	TCC	CAG	GTT	CTG	TTC	AAA	GGC	270
76	S	E	G	L		L	I	Y	S	Q	V	L	F	K	G	90
271	CAG	GGC	TGC	CCG	TCC	ACC	CAC	GTT	CTG	CTG	ACC	CAC	ACC	ATC	TCT	315
91	Q	G	C		S	T	H	V	L	L	T	H	T	I	S	105
316	CGT	ATC	GCT	GTT	TCC	TAC	CAG	ACC	aaa	GTA	AAC	CTG	CTG	TCT	GCA	360
106	R		A	V	S	Y	Q	T	K	V	N	L	L	S	A	120
361 121	ATC	AAA K	TCT	CCG	TGC C	CAG Q	CGT R	gaa e	ACC T	CCG P	gaa E	GGT G	GCT A	gaa E	GCT A	405 135
406	AAA	CCG	TGG	TAC	gaa	CCG	ATC	TAC	CTG	GGT	GGC	GTT	TTT	CAA	CTG	450
136	K	P	W	Y	E	P	I	Y	L	G	G	V	F	Q	L	150
451	GAG	AAA	GGT	GAC	CGT	CTG	TCT	GCA	GAA	ATT	AAC	CGT	CCG	GAC	TAC	495
151	E	K	G	D	R	L	S	A	E	I	N	R	P	D	Y	165
496	CTG	GAC	TTC	GCA	GAA	TCT	GGT	CaG	GTT	TAC	TTC	GGT	ATC	ATC	GCT	540
166	L	D	F	A	E	S	G	Q	V	Y	F	G	I	I	A	180
541	CTG	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCC	TCT	TCT	585
181	L	G	G	G	S	G	G	G	S	G	G	G	S	S	S	195
586	CGT	ACC	CCG	TCT	GAC	AAA	CCG	GTT	GCT	CAC	GTT	GTT	GCA	AAC	CCG	630
196	R	T	P	S	D	K	P	V	A	H	V	V	A	N	P	210
631	CAG	GCT	gaa	GGT	CAA	CTG	CAA	TGG	CTG	AAC	CGT	CGT	GCT	AAC	GCT	675
211	Q	A	E	G	Q	L	Q	₩	L	N	R	R	A	N	A	225
676	CTG	CTG	GCT	AAC	GGT	GTT	GAA	CTG	CGT	GAC	AAC	CAG	CTG	GTT	GTT	720
226	L	L	A	N	G	V	E	L	R	D	N	Q	L	V	V	240
721	CCG	TCT	gaa	GGC	CTG	TAC	CTG	ATC	TAC	TCC	CAG	GTT	CTG	TTC	AAA	765
241	P	S	E	G	L	Y	L	I	Y	S	Q	V	L	F	K	255
766 256	GGC G	CAG Q	GGC '	TGC C	CCG P	TCC :	ACC T	CAC H	GTT V	CTG L	CTG L	ACC T	CAC H	ACC T	ATC I	810 270

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								22/4	1							
Continuation																
811 271	TCI	CGT R	ATC	GCT A	GTT V	TCC	TAC	CAG	ACC T	AAA K	GTA V	AAC N	CTG L	CTG L	TCT S	855 285
856 286	GC#	ATC	AAA K	TCT	CCG	TGC	CAG Q	CGT R	GAA E	ACC T	CCG P	GAA E	GGT G	GCT A	gaa E	900 300
901 301	GCI A	AAA K	CCG	TGG W	TAC Y	GAA E	CCG	ATC	TAC Y	CTG L	GGT G	GGC G	GTT V	TTT F	CAA Q	945 315
946	CTG	GAG	AAA	GGT	GAC	CGT	CTG	TCT	GCA	GAA	ATT	AAC	CGT	CCG	GAC	990
316	L	E	K	G	D	R	L		A	E	I	N	R	P	D	330
991	TAC	CTG	GAC	TTC	GCA	GAA	TCT	GGT	CAG	GTT	TAC	TTC	GGT	ATC	ATC	1035
331	Y	L	D	F	A	E	S	G	Q	V	Y	F	G	I	I	345
1036	GCT	CTG	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCC	TCT	1080
346	A	L	G	G	G	S	G	G	G	S	G	G	G	S	S	360
1081	TCT	CGT	ACC	CCG	TCT	GAC	AAA	CCG	GTT	GCT	CAC	GTT	GTT	GCA	AAC	1125
361	S	R	T	P	S	D	K	P	V	A	H	V	V	A	N	375
1126	CCG	CAG	GCT	gaa	GGT	CAA	CTG	CAA	TGG	CTG	AAC	CGT	CGT	GCT	AAC	1170
376	P	Q	A	E	G	Q	L	Q	W	L	N	R	R	A	N	390
1171	GCT	CTG	CTG	GCT	AAC	GGT	GTT	GAA	CTG	CGT	GAC	AAC	CAG	CTG	GTT	1215
391	A	L	L	A	N	G	V	E	L	R	D	N	Q	L	V	405
1216	GTT	CCG	TCT	gaa	GGC	CTG	TAC	CTG	ATC	TAC	TCC	CAG	GTT	CTG	TTC	1260
406	V	P	S	E	G	L	Y	L	I	Y	S	Q	V	L	F	420
1261	AAA	GGC	CAG	GGC	TGC	CCG	TCC	ACC	CAC	GTT	CTG	CTG	ACC	CAC	ACC	1305
421	K	G	Q	G	C	P	S	T	H	V	L	L	T	H	T	435
1306	ATC	TCT	CGT	ATC	GCT	GTT	TCC	TAC	CAG	ACC	AAA	GTA	AAC	CTG	CTG	1350
436	I	S	R	I	A	V	S	Y	Q	T	K	V	N	L	L	450
1351	TCT	GCA	ATC	AAA	TCT	CCG	TGC	CAG	CGT	gaa	ACC	CCG	GAA	GGT	GCT	1395
451	S	A	I	K	S	P	C	Q	R	E	T	P	E	G	A	465
1396	GAA	GCT	AAA	CCG	TGG	TAC	gaa	CCG	ATC	TAC	CTG	GGT	GGC	G T T	TTT	1440
466	E	A	K	P	W	Y	E	P	I	Y	L	G	G	V	F	480
1441	CAA	CTG	GAG	AAA	GGT	GAC	CGT	CTG	TCT	GCA	gaa	ATT	AAC	CGT	CCG	1485
481	Q	L	E	K	G	D	R	L	S	A	E	I	N	R	P	495
1486	GAC	TAC	CTG	GAC	TTC	GCA	GAA	TCT	GGT	CAG	GTT	TAC	TTC	GGT	ATC	1530
496	D	Y	L	D	F	A	E	S	G	Q	V	Y	F	G	I	510
1531 511	ATC I	GCT A	CTG L	TGA *												1542 513

23/41 Figure 21

Nucleic acid sequence and corresponding amino acid sequence of scFasL Construct C

1	ATO M	GC:	T AT	C ATC	TAC Y	CTC L	ATC I	L	CTC L	TTC F	ACC T	GCT	GTG V	CGG R	GGC G	45 15
46 16	GC6 A	GC(GC(G GAT	TAC	AAA K	GAC D	GAT D	GAC D	GAT D	AAA K	GAA E	TTC	ACG T	CGT R	90 30
91 31	GAA E	AAI K	A AAC	G GAG	CTG L	AGG R	AAA K	GTG V	GCC A	CAT	TTA L	ACA T	GGC G	AAG K	TCC S	135 45
136 46	AAC N	TCA	AGG R	TCC S	ATG M	CCI	CTG L	GAA E	TGG W	GAA E	GAC D	ACC T	TAT Y	GGA G	ATT I	180 60
181 61	GTC V	CTG L	CTI L	TCT	GGA G	GTG V	AAG K	TAT	AAG K	AAG K	GGT G	GGC G	CTT L	GTG V	ATC I	225 75
226 76	AAT N	GAA E	ACT T	G G G	CTG L	TAC	TTT F	GTA V	TAT Y	TCC S	AAA K	GTA V	TAC Y	TTC F	CGG R	270 90
271 91	GGT G	CAA Q	TCT	TGC C	AAC N	AAC N	CTG L	CCC	CTG L	AGC S	CAC H	AAG K	GTC V	TAC Y	ATG M	315 105
316	AGG	AAC	TCT	AAG	TAT	CCC	CAG	GAT	CTG	GTG	ATG	ATG	GAG	GGG	AAG	360
106	R	N	S	K	Y		Q	D	L	V	M	M	E	G	K	120
361	ATG	ATG	AGC	TAC	TGC	ACT	ACT	GGG	CAG	ATG	TGG	GCC	CGC	AGC	AGC	405
121	M	M	S	Y	C	T	T	G	Ω	M	W	A	R	S	S	135
406	TAC	CTG	GGG	GCA	GTG	TTC	AAT	CTT	ACC	AGT	GCT	GAT	CAT	TTA	TAT	450
136	Y	L	G	A	V	F	N	L	T	S	A	D	H	L	Y	150
451	gtc	AAC	GTA	TCT	GAG	CTC	TCT	CTG	GTC	AAT	TTT	gag	gaa	TCT	CAG	495
151	V	N	V	S	E	L	S	L	V	N	F	E	E	S	Q	165
496	ACG	TTT	TTC	GGC	TTA	TAT	AAG	CTC	GGT	GGC	GGT	TCT	GGT	GGC	GGT	540
166	T	F	F	G	L	Y	K	L	G	G	G	S	G	G	G	180
541	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	GAA	AAA	AAG	gag	CTG	AGG	585
181	S	G	G	G	S	G	G	G	S	E	K	K	E	L	R	195
586	AAA	GTG	GCC	CAT	TTA	ACA	GGC	AAG	TCC	AAC	TCA	AGG	TCC	ATG	CCT	630
196	K	V	A	H	L	T	G	K	S	N	S	R	S	M		210
631	CTG	GAA	TGG	GAA	GAC	ACC	TAT	GGA	ATT	GTC	CTG	CTT	TCT	GGA	GTG	675
211	L	E	W	E	D	T	Y	G	I	V	L	L	S	G	V	225
676	AAG	TAT	AAG	AAG	GGT	GGC	CTT	GTG	ATC	AAT	GAA	ACT	GGG	CTG	TAC	720
226	K	Y	K	K	G	G	L	V	I	N	E	T	G	L	Y	240
721 241	TTT (GTA V	TAT Y	TCC :	AAA K	GTA V	TAC Y	TTC F	CGG R	GGT G	CAA Q	TCT S	TGC C	AAC N	AAC N	765 255

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Continuation of Figure 21 CTG CCC CTG AGC CAC AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC 256 LPLSHKVYMRNSKYP 270 CAG GAT CTG GTG ATG ATG GAG GGG AAG ATG ATG AGC TAC TGC ACT 855 811 271 Q D L V M M E G K M M S Y C T 285 856 ACT GGG CAG ATG TGG GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC 900 286 T G O M W A R S S Y L 300 AAT CTT ACC AGT GCT GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC 945 901 S A D H L Y v N 17 315 990 946 TCT CTG GTC AAT TTT GAG GAA TCT CAG ACG TTT TTC GGC TTA TAT SLVNFEESOTFFGLY 330 316 991 1035 331 G G S G G G S G G G S G 345 1036 GGC GGA TCC GAA AAA AAG GAG CTG AGG AAA GTG GCC CAT TTA ACA 1080 G G S EKKELRKVAHL 360 1081 GGC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC 1125 N S R S M E 375 1126 TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG TAT AAG AAG GGT GGC 1170 376 V L L SGVKY K K G G 390 1171 CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA 1215 Y T, V T NETGI F V Y S к 405 391 1216 TAC TTC CGG GGT CAA TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG 1260 FRGOSCNNLP 420 406 1261 GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG 1305 421 V Y M R N S K Y P ODIVMM 435 1306 GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG GCC 1350 E G K M M S Y C T T G O M W A 1351 CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT CTT ACC AGT GCT GAT 1395 Y L G A V F N L T S 465 1396 CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT GAG 1440 v N V S E L SL V N F E 480 v 1441 GAA TOT CAG ACG TTT TTC GGC TTA TAT AAG CTC TGA 1476 ESOTFFGLYKL 491

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Figure 22
Nucleic acid sequence and corresponding amino acid sequence of scTRAIL Construct D

1	ATC M	G GCT	T ATC	ATC I	TAC Y	CTC L	ATC I	L	CTG L	TTC F	ACC T	GCT A	GTG V	CGG R	GGC G	45 15
46 16	GCG A	G GCC	GCG A	GAT D	TAC	AAA K	GAC D	GAT D	GAC D	GAT D	AAA K	GAA E	TTC F	GGA G	ACC T	90 30
91 31	TCT	GAG E	GAA E	ACC T	ATT I	TCT	ACA T	GTT V	CAA Q	GAA E	AAG K	CAA Q	CAA Q	AAT N	ATT I	135 45
136	TCT	CCC	CTA	GTG	AGA	GAA	AGA	GGT	CCT	CAG	AGA	GTA	GCA	GCT	CAC	180
46	S		L	V	R	E	R	G	P	Q	R	V	A	A	H	60
181	ATA	ACT	GGG	ACC	AGA	GGA	AGA	AGC	AAC	ACA	TTG	TCT	TCT	CCA	AAC	225
61	I		G	T	R	G	R	S	N	T	L	S	S	P	N	75
226	TCC	AAG	AAT	GAA	AAG	GCT	CTG	GGC	CGC	AAA	ATA	AAC	TCC	TGG	GAA	270
76		K	N	E	K	A	L	G	R	K	I	N	S	W	E	90
271	TCA	TCA	AGG	AGT	GGG	CAT	TCA	TTC	CTG	AGC	AAC	TTG	CAC	TTG	AGG	315
91	S	S	R	S	G	H	S	F	L	S	N	L	H	L	R	105
316	AAT	GGT	gaa	CTG	GTC	ATC	CAT	gaa	AAA	GGG	TTT	TAC	TAC	ATC	TAT	360
106	N	G	E	L	V	I	H	E	K	G	F	Y	Y	I	Y	120
361	TCC	CAA	ACA	TAC	TTT	CGA	TTT	CAG	GAG	gaa	ATA	AAA	gaa	AAC	ACA	405
121	S	Q	T	Y	F	R	F	Q	E	E	I	K	e	N	T	135
406	AAG	AAC	GAC	AAA	CAA	ATG	GTC	CAA	TAT	ATT	TAC	AAA	TAC	ACA	AGT	450
136	K	N	D	K	Q	M	V	Q	Y	I	Y	K	Y	T	S	150
451	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	GCT	AGA	AAT	AGT	TGT	495
151	Y	P	D	P	I	L	L	M	K	S	A	R	N	S	C	165
496	TGG	TCT	AAA	GAT	gca	gaa	TAT	GGA	CTC	TAT	TCC	ATC	TAT	CAA	GGG	540
166	W	S	K	D	A	E	Y	G	L	Y	S	I	Y	Q	G	180
541	GGA	ATA	TTT	GAG	CTT	AAG	gaa	AAT	GAC	AGA	ATT	TTT	GTT	TCT	GTA	585
181	G	I	F	E	L	K	E	N	D	R	I	F	V	S	V	195
586	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	gaa	GCC	AGT	TTT	TTC	630
196	T	N	E	H	L	I	D	M	D	H	E	A	S	F	F	210
631	GGG	GCC	TTT	TTA	GTT	GGC	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	675
211	G	A	F	L	V	G	G	G	G	S	G	G	G	S	G	225
676	GGC	GGT	TCT	GGT	GGC	GGA	TCA	ACC	TCT	GAG	GAA	ACC	ATT	TCT	ACA	720
226	G	G	S	G	G	G	S	T	S	E	E	T	I	S	T	240
721	GTT	CAA	GAA	AAG	CAA	CAA .	AAT .	ATT	TCT	CCC	CTA	GTG	AGA	gaa	AGA	765
241	V	Q	E	K	Q		N	I	S	P	L	V	R	E	R	255

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Continuation of Figure 22 766 GGT CCT CAG AGA GTA GCA GCT CAC ATA ACT GGG ACC AGA GGA AGA 810 G P O R V A A H I T G T R G R 270 AGC AAC ACA TTG TCT TCT CCA AAC TCC AAG AAT GAA AAG GCT CTG 855 811 271 L S S P N S 285 856 GGC CGC AAA ATA AAC TCC TGG GAA TCA TCA AGG AGT GGG CAT TCA 900 286 G R K I N S W E S S R S G H S 300 TTC CTG AGC AAC TTG CAC TTG AGG AAT GGT GAA CTG GTC ATC CAT 945 301 SNLHLRNGELVIH 315 GAA AAA GGG TTT TAC TAC ATC TAT TCC CAA ACA TAC TTT CGA TTT 990 946 316 EKGFYYIYSOTYFRF 330 991 CAG GAG GAA ATA AAA GAA AAC ACA AAG AAC GAC AAA CAA ATG GTC 1035 Q E E I K E N T K N D K Q M V 1036 CAA TAT ATT TAC AAA TAC ACA AGT TAT CCT GAC CCT ATA TTG TTG 1080 K Y T S Y P 360 1081 ATG AAA AGT GCT AGA AAT AGT TGT TGG TCT AAA GAT GCA GAA TAT 1125 M K S A R N S C W S K D A E Y 375 1126 GGA CTC TAT TCC ATC TAT CAA GGG GGA ATA TTT GAG CTT AAG GAA 1170 G L Y S I Y Q G G I 376 FELKE 390 1171 AAT GAC AGA ATT TTT GTT TCT GTA ACA AAT GAG CAC TTG ATA GAC 1215 N D R I F V S V T N E H L I D 405 1216 ATG GAC CAT GAA GCC AGT TTT TTC GGG GCC TTT TTA GTT GGC GGT 1260 M D H E A S FFGAF L V G G 420 1305 G G G S G G G S G G 435 1306 ACC TCT GAG GAN ACC ATT TCT ACA GTT CAA GAA AAG CAA CAA AAT 1350 T S E E T I S T V Q E K Q Q N 450 436 1351 ATT TCT CCC CTA GTG AGA GAA AGA GGT CCT CAG AGA GTA GCA GCT 1395 451 S P L V R E R G P O R V 465 1396 CAC ATA ACT GGG ACC AGA GGA AGA AGC AAC ACA TTG TCT TCT CCA 1440 466 H I T G T R G R S N T L S 480 1441 AAC TCC AAG AAT GAA AAG GCT CTG GGC CGC AAA ATA AAC TCC TGG N S K N E K A L G R K I N 495 1486 GAA TCA TCA AGG AGT GGG CAT TCA TTC CTG AGC AAC TTG CAC TTG 1530 s G H S Τ. s N 510 1531 AGG AAT GGT GAA CTG GTC ATC CAT GAA AAA GGG TTT TAC TAC ATC RNGELVIHEKGFY 1576 TAT TCC CAA ACA TAC TTT CGA TTT CAG GAG GAA ATA AAA GAA AAC 1620 Y S O T Y F R F O E E I K E N 540

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Continuation	of Figure	22

1621	ACA	AAG	AAC	GAC	AAA	CAA	ATG	GTC	CAA	TAT	ATT	TAC	AAA	TAC	ACA	1665
541	T	K	N	D	K	Q	M	V	Q	Y	I	Y	K	Y	T	555
1666	AGT	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	GCT	AGA	AAT	AGT	1710
556	S	Y	P	D	P	I	L	L	M	K	S	A	R	N	S	570
1711	TGT	TGG	TCT	AAA	GAT	GCA	GAA	TAT	GGA	CTC	TAT	TCC	ATC	TAT	CAA	1755
571	C	W	S	K	D	A	E	Y	G	L	Y	S	I	Y	Q	585
1756	GGG	GGA	ATA	TTT	gag	CTT	AAG	gaa	AAT	GAC	AGA	ATT	TTT	g t t	TCT	1800
586	G	G	I	F	E	L	K	E	N	D	R	I	F	V	S	600
1801	gta	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	gaa	GCC	AGT	TTT	1845
501	V	T	N	E	H	L	I	D	M	D	H	E	A	S	F	615
1846 516	TTC F	GGG G	GCC A	TTT F	TTA L	GTT V	GGC G	TGA *								1866 622

28/41 Figure 23

Nucleic acid sequence and corresponding amino acid sequence of scTNF Construct E

ATG GCT ATC ATC TAC CTC ATC CTC CTG TTC ACC GCT GTG CGG GGC 1 45 1 L GCG GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA GAA TTC GGA TCA 90 v ĸ n n D n K E 30 TCT TCT CGA ACC CCG AGT GAC AAG CCT GTA GCC CAT GTT GTA GCA 135 91 А 31 AAC CCT CAA GCT GAG GGG CAG CTC CAG TGG CTG AAC CGC CGG GCC 136 180 46 150 G Q L Q W L 60 181 AAT GCC CTC CTG GCC AAT GGC GTG GAG CTG AGA GAT AAC CAG CTG 225 Α N G 10 T. R 75 61 226 GTG GTG CCA TCA GAG GGC CTG TAC CTC ATC TAC TCC CAG GTC CTC 270 E G L Y L 76 I 90 271 TTC AAG GGC CAA GGC TGC CCC TCC ACC CAT GTG CTC CTC ACC CAC 315 G C P S T H V L L 91 105 ACC ATC AGC CGC ATC GCC GTC TCC TAC CAG ACC AAG GTC AAC CTC 360 316 120 106 361 CTC TCT GCC ATC AAG AGC CCC TGC CAG AGG GAG ACC CCA GAG GGG 405 121 I K S P C O R E 135 GCT GAG GCC AAG CCC TGG TAT GAG CCC ATC TAT CTG GGA GGG GTC 450 406 ĸ 53 v F 150 136 P I Y TTC CAG CTG GAG AAG GGT GAC CGA CTC AGC GCT GAG ATC AAT CGG 451 495 D 165 151 G R CCC GAC TAT CTC GAC TTT GCC GAG TCT GGG CAG GTC TAC TTT GGG 540 496 166 n Δ E S G 0 180 585 541 181 G G s G G G S 195 TCT GGT GGC GGA TCA TCT TCT CGA ACC CCG AGT GAC AAG CCT 630 196 S s R т 210 GTA GCC CAT GTT GTA GCA AAC CCT CAA GCT GAG GGG CAG CTC CAG 675 631 Δ N O A 763 225 211 676 TGG CTG AAC CGC CGG GCC AAT GCC CTC CTG GCC AAT GGC GTG GAG 720 226 Α Α 240 721 CTG AGA GAT AAC CAG CTG GTG GTG CCA TCA GAG GGC CTG TAC CTC 765 L R D N Q L V V P S E G L Y L 241 255

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Continuation of Fig	gure	23
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766 256	ATO	TAC Y	TO	C CAC	GTC V	CTC L	F	K AAG	G GGC	CAF Q	G GGC	TGC	CCC	TCC S	ACC T	810 270
811 271	CAT H	r gre	CT(C CTC	ACC T	CAC H	ACC T	ATC I	AGO	CGC R	ATC I	GCC	GTC	TCC	TAC Y	855 285
856 286	CAG Q	ACC T	: AAC	G GTC	AAC N	CTC L	CTC L	TCT S	GCC	ATC	AAG K	AGC S	CCC	TGC C	CAG Q	900 300
901 301	AGC R	GAG E	ACC T	P CCA	GAG E	GGG G	GCT A	GAG E	GCC	AAG K	CCC	TGG W	TAT	GAG E	CCC P	945 315
946 316	ATO	TAT Y	CTG L	GGA G	GGG G	GTC V	TTC F	CAG Q	CTG L	GAG E	AAG K	GGT G	GAC D	CGA R	CTC L	990 330
991 331	AGC S	GCT A	GAG E	ATC	AAT N	CGG R	CCC	GAC D	TAT Y	CTC	GAC D	TTT F	GCC A	GAG E	TCT S	1035 345
1036 346	GGG G	CAG Q	GTC V	TAC	TTT F	GGG G	ATC	ATT	GCC A	CTG L	GGT G	GGC G	GGT G	TCT S	GGT G	1080 360
1081 361	GGC G	GGT	TCT S	GGT G	GGC G	GGT G	TCT S	GGT G	GGC G	GGA G	TCA S	TCA S	TCT S	TCT S	CGA R	1125 375
1126 376	ACC T	CCG P	AGT S	GAC D	AAG K	CCT P	GTA V	GCC A	CAT H		gta V	GCA A	AAC N	CCT P	CAA Q	1170 390
171 391	GCT A	GAG E	GGG G	CAG Q	CTC L	CAG Q	TGG W	CTG L	AAC N	CGC R	CGG R	GCC A	AAT N	GCC A	CTC L	1215 405
1216 106	CTG L	GCC A	AAT N	GGC G	GTG V	GAG E	CTG L	AGA R	GAT D	AAC N	CAG Q	CTG L	GTG V	g t g V	CCA P	1260 420
261 121	TCA S	GAG E	GGC G	CTG L	TAC Y	CTC L	ATC I	TAC Y	TCC S	CAG Q	GTC V	CTC L	TTC F	AAG K	GGC G	1305 435
306 36	CAA Q	GGC G	TGC	CCC P	TCC	ACC T	CAT H	gtg V	CTC L	CTC L	ACC T	CAC H	ACC T	ATC I	AGC S	1350 450
351 51	CGC R	ATC	GCC A	GTC V	TCC	TAC Y	CAG Q	ACC T	AAG K	GTC V	AAC N	CTC L	CTC L	TCT S	GCC A	1395 465
396 66	ATC I	AAG K	AGC S	P	TGC C	CAG Q	AGG R	GAG E	ACC T	CCA P	gag E	GGG G	GCT A	GAG E	GCC A	1440 480
441 81	AAG K	CCC	TGG W	TAT Y	GAG E	CCC	ATC I	TAT Y	CTG L	GGA G	GGG G	GTC V	TTC F	CAG Q	CTG L	1485 495
486 96	GAG E	AAG K	GGT G	GAC D	CGA R	CTC L	AGC S	GCT A	GAG E	ATC I	AAT N	CGG R	CCC	GAC D	TAT Y	1530 510
531 11	CTC L	GAC D	TTT F	GCC A	GAG E	TCT S	GGG G	CAG Q	GTC V	TAC Y	TTT F	GGG G	ATC I	ATT	GCC A	1575 525
576 26	CTG L															1581 526

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Figure 24
Nucleic acid sequence and corresponding amino acid sequence of scFasL-AMAIZe
Construct F

1	ATG	GAC	TGG	ACC	TGG	CGC	GTG	TTT	TGC	CTG	CTC	GCC	GTG	GCT	CCT	45
	M	D	W	T	W	R	V	F	C	L	L	A	V	A	P	15
46	GGG	GCC	CAC	AGC	CAG	GTA	CAG	CTG	gTG	CAG	TCT	GGG	GGA	GGC	ATG	90
16	G		H	S	Q	V	Q	L	V	Q	S	G	G	G	M	30
91	GTA	GAG	CCT	GGG	GGG	TCC	CTT	AGA	CTC	TCC	TGT	GCA	GCC	TCT	GGA	135
31	V	E		G	G	S	L	R	L	S	C	A	A	S	G	45
136	TTC	ACT	TTC	AGT	aat	GCC	TGG	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	180
46	F	T	F	S	N	A	W	M	S	W	V	R	Q	A	P	60
181	GGG	AAG	GGG	CTG	GAG	TGG	GTT	GGC	CGT	ATA	AAA	AGC	AAA	GCT	GGT	225
61	G	K	G	L	E	W	V	G	R	I	K	S	K	A	G	75
226	GGT	GGG	ACA	GCA	GAG	TAC	GCT	GCA	CCC	GTG	aaa	GGC	AGA	TTC	ACC	270
76	G	G	T	A	E	Y	A	A	P	V	K	G	R	F	T	90
271	ATC	TCA	AGA	GAT	GAT	TCA	CAA	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	315
91	I	S	R	D	D	S	Q	N	T	L	Y	L	Q	M	N	105
316	AGC	CTG	AAA	ACC	GAC	GAC	ACA	GCC	GTG	TAT	TAC	TGT	ACC	ACA	CAT	360
106	S	L	K	T	D	D	T	A	V	Y	Y	C	T	T	H	120
361	GTC	TAC	GGT	GCC	CCC	CGG	AAC	TGG	GGC	CAG	GGA	TCC	CTG	GTC	ACC	405
121	V	Y	G	A	P	R	N	W	G	Q	G	S	L	V	T	135
406	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	AAG	CTT	gaa	gaa	GGT	GAA	450
136	V	S	S	A	S	T	K	G	P	K	L	E	E	G	E	150
451	T TT	TCA	GAA	GCA	CGC	GTA	CAG	TCT	GTG	TTG	ACT	CAG	CCG	CCC	TCA	495
151	F	S	E	A	R	V	Q	S	V	L	T	Q	P	P	S	165
496	gTG	TCT	GCG	GCC	CCA	GGA	CAG	aag	GTC	ACC	ATC	TCC	TGC	TCT	GGA	540
166	V	S	A	A	P	G	Q	K	V	T	I	S	C	S	G	180
541	AGC	AGC	TCC	AAC	ATT	GGA	AAT	AAT	TAT	GTC	TCC	TGG	TAC	GTT	CAA	58 5
181	S	S	S	N		G	N	N	Y	V	S	₩	Y	V	Q	195
586	CTC	CCA	GGA	ACA	GCC	CCC	AAA	CTC	CTC	ATT	TAT	GAC	AAT	AAT	AAG	630
196	L	P	G	T	A	P	K	L	L		Y	D	N	N	K	210
631	CGA	TTC	TCA	GGA	GTT	CCT	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	675
211	R	F	S	G	V	P	D	R	F	S	G	S	K	S	G	225
676	ACG	TCA	GCC	ACC	CTG	GGC	ATC	ACC	GGG	CTC	CAG	ACT	GGG	GAC	GAG	720
226	T	S	A	T	L	G	I	T	G	L	Q	T	G	D	E	240
721	GCC	GAT	TAT	TAC	TGC	GGA	GCA	TGG	GAT	GGC	AGC	CTG	CGT	GAA	GCG	765
241	A	D	Y	Y	C	G	A	W	D	G	S	L	R	E	A	255

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Continuation of Figure 24 GTA TTC GGC GGA GGG ACC AAG GTC ACC GTC CTA GGT GCG GCC GCA 810 256 V F G G G T K V T V L G A A A 270 811 GTT GAG CTC GAG gcg GCC GCG GAT TAC AAA GAC GAT GAC GAT AAA 855 271 VELEAAAD Y K D 285 856 GAA TTC ACG CGT GAA AAA AAG GAG CTG AGG AAA GTG GCC CAT TTA 900 286 EFTREKKELRK 300 ACA GGC AAG TCC AAC TCA AGG TCC ATG CCT CTG GAA TGG GAA GAC 901 301 K S N S R S M 315 ACC TAT GGA ATT GTC CTG CTT TCT GGA GTG AAG TAT AAG AAG GGT 946 990 316 TYGIVLLSGVKYKKG 330 GGC CTT GTG ATC AAT GAA ACT GGG CTG TAC TTT GTA TAT TCC AAA 991 1035 VINETGLYFVYSK 331 345 1036 GTA TAC TTC CGG GGT CAA TCT TGC AAC AAC CTG CCC CTG AGC CAC 1080 346 G Q S C N N 360 1081 AAG GTC TAC ATG AGG AAC TCT AAG TAT CCC CAG GAT CTG GTG ATG 1125 361 Y M R N S K Y P Q D L V M 375 1126 ATG GAG GGG AAG ATG ATG AGC TAC TGC ACT ACT GGG CAG ATG TGG 1170 376 MEGKMM SYCT T G Q M W 390 1171 GCC CGC AGC AGC TAC CTG GGG GCA GTG TTC AAT CTT ACC AGT GCT 1215 391 ARSSYLGAVFNLTSA 405 1216 GAT CAT TTA TAT GTC AAC GTA TCT GAG CTC TCT CTG GTC AAT TTT 1260 406 HLYVNVSEL SLVNF 420 1261 GAG GAA TCT CAG ACG TTT TTC GGC TTA TAT AAG CTC GGT GGC GGT 1305 QTFFGLYKL G 435 1350 G G G S G G G G s E 450 1351 AAG GAG CTG AGG AAA GTG GCC CAT TTA ACA GGC AAG TCC AAC TCA 1395 ELRKVAHLTGKSN 465 1396 AGG TCC ATG CCT CTG GAA TGG GAA GAC ACC TAT GGA ATT GTC CTG 1440 L E W E D T Y G 480 1441 CTT TCT GGA GTG AAG TAT AAG AAG GGT GGC CTT GTG ATC AAT GAA 1485 LSGVKYKKGGLVIN 495 1486 ACT GGG CTG TAC TTT GTA TAT TCC AAA GTA TAC TTC CGG GGT CAA 1530 YFV v S K V Y F R 510 1531 TCT TGC AAC AAC CTG CCC CTG AGC CAC AAG GTC TAC ATG AGG AAC 1575 S C N N L P L S H K V Y M R 525 1576 TCT AAG TAT CCC CAG GAT CTG GTG ATG ATG GAG GGG AAG ATG ATG 1620

S K Y P Q D L V M M E G K M M

540

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Continuation	of Figure	24

1621	AGC	TAC	TGC	ACT	ACT	GGG	CAG	ATG	TGG	GCC	CGC	AGC	AGC	TAC	CTG	1665
541	S	Y	C	T	T	G	Q	M	W	A	R	S	S	Y	L	555
1666	GGG	GCA	GTG	TTC	AAT	CTT	ACC	AGT	GCT	GAT	CAT	TTA	TAT	GTC	AAC	1710
556	G	A	V	F	N	L	T	S	A	D	H	L	Y	V	N	570
1711	gta	TCT	GAG	CTC	TCT	CTG	GTC	AAT	TTT	GAG	GAA	TCT	CAG	ACG	TTT	1755
571	V	S	E	L	S	L	V	N	F	E	E	S	Q	T	F	585
1756	TTC	GGC	TTA	TAT	AAG	CTC	GGT	GGC	GĢT	TCT	GGT	GGC	GGT	TCT	GGT	1800
586	F	G	L	Y	K	L	G	G	G	S	G	G	G	S	G	600
1801	GGC	GGT	TCT	GGT	GGC	GGA	TCC	gaa	AAA	AAG	GAG	CTG	AGG	AAA	gtg	1845
601	G	G	S	G	G	G	S	E	K	K	E	L	R	K	V	615
1846	GCC	CAT	TTA	ACA	GGC	AAG	TCC	AAC	TCA	AGG	TCC	ATG	CCT	CTG	GAA	1890
616	A	H	L	T	G	K	S	N	S	R	S	M	P	L	E	630
1891	TGG	GAA	GAC	ACC	TAT	GGA	ATT	GTC	CTG	CTT	TCT	GGA	GTG	AAG	TAT	1935
631	W	E	D	T	Y	G	I	V	L	L	S	G	V	K	Y	645
1936	AAG	AAG	GGT	GGC	CTT	GTG	ATC	AAT	gaa	ACT	GGG	CTG	TAC	TTT	GTA	1980
646	K	K	G	G	L	V	I	N	E	T	G	L	Y	F	V	660
1981	TAT	TCC	AAA	GTA	TAC	TTC	CGG	GGT	CAA	TCT	TGC	AAC	AAC	CTG	CCC	2025
661	Y	S	K	V	Y	F	R	G	Q	S	C	N	N	L		675
2026	CTG	AGC	CAC	AAG	GTC	TAC	ATG	AGG	AAC	TCT	AAG	TAT	CCC	CAG	GAT	2070
676	L	S	H	K	V	Y	M	R	N	S	K	Y		Q	D	690
2071	CTG	GTG	ATG	ATG	GAG	GGG	AAG	ATG	ATG	AGC	TAC	TGC	ACT	ACT	GGG	2115
691	L	V	M	M	E	G	K	M	M	S	Y	C	T	T	G	705
2116	CAG	ATG	TGG	GCC	CGC	AGC	AGC	TAC	CTG	GGG	GCA	g t g	TTC	AAT	CTT	2160
706	Q	M	W	A	R	S	S	Y	L	G	A	V	F	N	L	720
2161	ACC	AGT	GCT	GAT	CAT	TTA	TAT	GTC	AAC	GTA	TCT	GAG	CTC	TCT	CTG	2205
721	T	S	A	D	H	L	Y	V	N	V	S	E	L	S	L	735
2206	GTC	AAT	TTT	GAG	gaa	TCT	CAG	ACG	TTT	TTC	GGC	TTA	TAT	AAG	CTC	2250
736	V	N	F	E	E	S	Q	T	F	F	G	L	Y	K	L	750
2251 751	TGA															2253 751

33/41 Figure 25

Nucleic acid sequence and corresponding amino acid sequence of scTRAIL-AMAIZe Construct G

1		ATG M	GAC D	TGG W	ACC T	TGG W	CGC R	GTG V	TTT F	TGC C	CTG L	CTC L	GCC A	GTG V	GCT A	CCT P	45 15
1		GGG G	GCC A	CAC H	AGC S	CAG Q	GTA V	CAG Q	CTG L	gtg V	CAG Q	TCT S	GGG G	GGA G	GGC G	ATG M	90 30
9: 3:		GTA V	GAG E	CCT	GGG G	GGG G	TCC S	CTT L	AGA R	CTC L	TCC S	TGT C	GCA A	GCC A	TCT S	GGA G	135 45
13	36	TTC	ACT	TTC	AGT	AAT	GCC	TGG	ATG	AGC	TGG	GTC	CGC	CAG	GCT	CCA	180
	6	F	T	F	S	N	A	W	M	S	W	V	R	Q	A	P	60
18		GGG	AAG	GGG	CTG	GAG	TGG	GTT	GGC	CGT	ATA	AAA	AGC	AAA	GCT	GGT	225
61		G	K	G	L	E	W	V	G	R	I	K	S	K	A	G	75
22 76		GGT G	GGG G	ACA T	GCA A	GAG E	TAC Y	GCT A	GCA A	CCC	GTG V	AAA K	GGC G	AGA R	TTC F	ACC T	270 90
27		ATC	TCA	AGA	GAT	GAT	TCA	CAA	AAC	ACG	CTG	TAT	CTG	CAA	ATG	AAC	315
91		I	S	R	D	D	S	Q	N	T	L	Y	L	Q	M	N	105
31		AGC	CTG	AAA	ACC	GAC	GAC	ACA	GCC	gtg	TAT	TAC	TGT	ACC	ACA	CAT	360
10		S	L	K	T	D	D	T	A	V	Y	Y	C	T	T	H	120
36		GTC	TAC	GGT	GCC	CCC	CGG	AAC	TGG	GGC	CAG	GGA	TCC	CTG	GTC	ACC	405
12		V	Y	G	A	P	R	N	W	G	Q	G	S	L	V	T	135
40		GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	AAG	CTT	GAA	GAA	GGT	GAA	450
13		V	S	S	A	S	T	K	G	P	K	L	E	E	G	E	150
45		TTT	TCA	gaa	GCA	CGC	gta	CAG	TCT	GTG	TTG	ACT	CAG	CCG	CCC	TCA	495
15		F	S	E	A	R	V	Q	S	V	L	T	Q	P	P	S	165
49		GTG	TCT	GCG	GCC	CCA	GGA	CAG	AAG	GTC	ACC	ATC	TCC	TGC	TCT	GGA	540
16		V	S	A	A	P	G	Q	K	V	T	I	S	C	S	G	180
54		AGC	AGC	TCC	AAC	ATT	GGA	AAT	AAT	TAT	GTC	TCC	TGG	TAC	GTT	CAA	585
18		S	S	S	N	I	G	N	N	Y	V	S	W	Y	V	Q	195
58		CTC	CCA	GGA	ACA	GCC	CCC	AAA	CTC	CTC	ATT	TAT	GAC	AAT	AAT	AAG	630
19		L	P	G	T	A	P	K	L	L	I	Y	D	N	N	K	210
63		CGA	TTC	TCA	GGA	GTT	CCT	GAC	CGA	TTC	TCT	GGC	TCC	AAG	TCT	GGC	675
21		R	F	S	G	V	P	D	R	F	S	G	S	K	S	G	225
67 22		ACG	TCA S	GCC A	ACC T	CTG L	GGC .	ATC	ACC T	GGG G	CTC L	CAG Q	ACT T	GGG G	GAC D	GAG E	720 240

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Continuation of Figure 25

721 241	GC(GAT D	TAT Y	TAC Y	TGC	GGA G	GCA A	TGG W	GAT D	GGC G	AGC S	CTG L	CGT	GAA E	GCG A	765 255
766 256	GT <i>I</i> V	TTC	GGC G	GGA G	GGG G	ACC	AAG K	GTC V	ACC	GTC V	CTA L	GGT G	GCG A	GCC A	GCA A	810 270
811	GTT	GAG	CTC	GAG	GCG	GCC	GCG	GAT	TAC	AAA	GAC	GAT	GAC	GAT	AAA	855
271	V	E	L	E	A	A	A	D	Y	K	D	D	D	D	K	285
856 286	GAA E	TTC	GGA G	ACC	TCT	GAG E	gaa e	ACC T	ATT I	TCT	ACA T	GTT V	CAA Q	gaa E	AAG K	900 300
901	CAA	CAA	AAT	ATT	TCT	CCC	CTA	gtg	AGA	GAA	AGA	GGT	CCT	CAG	AGA	945
301	Q	Q	N	I	S	P	L	V	R	E	R	G	P	Q	R	315
946	GTA	GCA	GCT	CAC	ATA	ACT	GGG	ACC	AGA	GGA	AGA	AGC	AAC	ACA	TTG	990
316	V	A	A	H	I	T	G	T	R	G	R	S	N	T	L	330
991	TCT	TCT	CCA	AAC	TCC	AAG	AAT	gaa	AAG	GCT	CTG	GGC	CGC	AAA	ATA	1035
331	S	S	P	N	S	K	N	E	K	A	L	G	R	K	I	345
1036	AAC	TCC	TGG	gaa	TCA	TCA	AGG	AGT	GGG	CAT	TCA	TTC	CTG	AGC	AAC	1080
346	N	S	₩	E	S	S	R	S	G	H	S	F	L	S	N	360
1081	TTG	CAC	TTG	AGG	AAT	GGT	gaa	CTG	GTC	ATC	CAT	GAA	aaa	GGG	TTT	1125
361	L	H	L	R	N	G	E	L	V	I	H	E	K	G	F	375
1126	TAC	TAC	ATC	TAT	TCC	CAA	ACA	TAC	TTT	CGA	TTT	CAG	GAG	GAA	ATA	1170
376	Y	Y	I	Y	S	Q	T	Y	F	R	F	Q	E	E	I	390
1171	AAA	GAA	AAC	ACA	AAG	AAC	GAC	AAA	CAA	ATG	GTC	CAA	TAT	ATT	TAC	1215
391	K	E	N	T	K	N	D	K	Q	M	V	Q	Y	I	Y	405
1216	AAA	TAC	ACA	AGT	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	GCT	1260
406	K	Y	T	S	Y	P	D	P	I	L	L	M	K	S	A	420
1261	AGA	AAT	AGT	TGT	TGG	TCT	AAA	GAT	GCA	GAA	TAT	GGA	CTC	TAT	TCC	1305
421	R	N	S	C	W	S	K	D	A	E	Y	G		Y	S	435
1306	ATC	TAT	CAA	GGG	GGA	ATA	TTT	GAG	C T T	AAG	GAA	aat	GAC	AGA	ATT	1350
436	I	Y	Q	G	G	I	F	E		K	E	N	D	R	I	450
1351	TTT	GTT	TCT	GTA	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	GAA	1395
451	F	V	S	V	T	N	E	H	L	I	D	M	D	H	E	465
1396	GCC	AGT	TTT	TTC	GGG	GCC	TTT	TTA	GTT	GGC	GGT	GGC	GGT	TCT	GGT	1440
466	A	S	F	F	G	A	F	L	V	G	G	G	G	S	G	480
1441	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	ACC	TCT	GAG	GAA	1485
481	G	G	S	G	G	G	S	G	G	G	S	T	S	E	E	495
1486	ACC	ATT	TCT	ACA	GTT	CAA	gaa	AAG	CAA	CAA	AAT	ATT	TCT	CCC	CTA	1530
496	T	I	S	T	V	Q	E	K	Q	Q	N	I	S		L	510
1531 511	GTG V	AGA R	GAA.	AGA R	GGT G	CCT	CAG Q	AGA R	GTA V	GCA A	GCT A	CAC H	ATA I	ACT T	GGG G	1575 525

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Continuation of Figure 25

157 526			GA GG		A AG	C AAC N	ACA T	A TTO	TC:	r rcr	P CC	AAC N	TCC	C AAG	AAT N	1620 540
162 541	1 G/	A A	G GC	T CT	G GGG	C CGC	AA!	ATA A	A AAC N	TCC	TGC W	GAA	TCF	TCA S	AGG R	1665 555
166 556	6 AG		G CA		A TTO	CTG	AGO	AAC N	TTC L	CAC H	TTC L	AGG	AAT N	G G T	GAA E	1710 570
171 571	1 CT		C AT	C CAI	GAF	AAA K	GGG	TTI F	TAC	TAC	ATC	TAT	TCC	CAA Q	ACA T	1755 585
175 586	6 TA Y	C TT		A TT	CAG	GAG E	GAA E	ATA	AAA K	GAA E	AAC N	ACA T	AAG K	AAC N	GAC D	1800 600
1801 601	L AA K	A CA	A ATO	G GT C	CAA Q	TAT	ATT	TAC	AAA K	TAC	ACA T	AGT S	TAT	CCT	GAC D	1845 615
1846 616	p CC	r AT	A TTO	TTG L	ATG M	AAA K	AGT S	GCT A	AGA R	AAT N	AGT S	TGT C	TGG W	TCT	AAA K	1890 630
1891 631	GA'	P GC	A GAZ	TAT Y	GGA G	CTC L	TAT Y	TCC S	ATC	TAT Y	CAA Q	GGG G	GGA G	ATA I	TTT F	1935 645
1936 646	GA(CT.	r aac K	GAA E	AAT N	GAC D	AGA R	ATT	TTT F	GTT V	TCT S	GTA V	ACA T	AAT N	GAG E	1980 660
1981 661	CAC H	TTC L	ATA	GAC D	ATG M	GAC D	CAT H	GAA E	GCC A	AGT S	TTT F	TTC F	GGG G	GCC A	TTT F	2025 675
2026 676	TTA L	GT'	GGC G	GGT	GGC G	GGT G	TCT S	GGT G	GGC G	GGT G	TCT S	GGT G	GGC G	GGT G	TCT S	2070 690
2071 691	GGT G	G GG	G GGA	TCC	ACC T	TCT S	GAG E	gaa E	ACC T	ATT I	TCT S	ACA T	GTT V	CAA Q	GAA E	2115 705
2116 706	AAG K	CAA Q	CAA Q	AAT N	ATT I	TCT S	CCC	CTA L	GTG V	AGA R	GAA E	AGA R	GGT G	CCT P	CAG Q	2160 720
2161 721	AGA R	GTA V	GCA A	GCT A	CAC H	ATA .	ACT T	GGG G	ACC T	AGA R	GGA G	AGA R	AGC S	AAC N	ACA T	2205 735
2206 736	TTG L	TCT	TCT S	CCA P	AAC N	TCC .	AAG K	aat N	GAA E	AAG K	GCT A	CTG L	GGC G	CGC R	AAA K	2250 750
2251 751	ATA I	AAC N	TCC S	TGG W	gaa E	TCA S	TCA S	AGG R	AGT S	GGG G	CAT H	TCA S	TTC F	CTG L	AGC S	2295 765
2296 766	AAC N	TTG L	CAC H	TTG L	AGG R	AAT (G G	GAA E	CTG L	GTC V	ATC I	CAT H	GAA E	AAA K	GGG G	2340 780
2341 781	TTT F	TAC Y	TAC Y	ATC I	TAT Y	TCC (CAA Q	ACA T	TAC Y	TTT F	CGA R	TTT F	CAG Q	GAG E	GAA E	2385 795
2386 796	ATA I	AAA K	GAA E	AAC N	ACA T	AAG A	N N	GAC D	AAA K	CAA Q	ATG M	GTC V	CAA Q	TAT Y	ATT I	2430 810

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								JU/4								
Continuation	n of Fi	gure	25													
243		AAA	TAC	ACA	AGT	TAT	CCT	GAC	CCT	ATA	TTG	TTG	ATG	AAA	AGT	2475
811		K	Y	T	S	Y	P	D	P	I	L	L	M	K	S	825
247		AGA	AAT	AGT	TGT	TGG	TCT	AAA	GAT	GCA	GAA	TAT	GGA	CTC	TAT	2520
826		R	N	S	C	W	S	K	D	A	E	Y	G	L	Y	840
252		ATC	TAT	CAA	GGG	GGA	ATA	TTT	GAG	CTT	AAG	GAA	AAT	GAC	AGA	2565
841		I	Y	Q	G	G	I	F	E	L	K	E	N	D	R	855
256		TTT	GTT	TCT	GTA	ACA	AAT	GAG	CAC	TTG	ATA	GAC	ATG	GAC	CAT	2610
856		F	V	S	V	T	N	E	H	L	I	D	M	D	H	870
261 871	1 GAA E	GCC A	AGT S	TTT F	TTC F	GGG G	GCC A	TTT F	TTA L	GTT V	GGC G	TGA *				2646 881

37/41 Figure 26 Nucleic acid sequence and corresponding amino acid sequence of scTNF-AMAIZe

Construct H ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC GTG GCT CCT 45 1 R v F C L L A V A 1 15 GGG GCC CAC AGC CAG GTA CAG CTG GTG CAG TCT GGG GGA GGC ATG 90 s Q 0 L v 0 S G 30 91 GTA GAG CCT GGG GGG TCC CTT AGA CTC TCC TGT GCA GCC TCT GGA 135 G 31 S L R ь S C Α 45 136 TTC ACT TTC AGT AAT GCC TGG ATG AGC TGG GTC CGC CAG GCT CCA 180 S N A W M S wv 46 R 60 181 GGG AAG GGG CTG GAG TGG GTT GGC CGT ATA AAA AGC AAA GCT GGT 225 Ξ W V G R K 75 GGT GGG ACA GCA GAG TAC GCT GCA CCC GTG AAA GGC AGA TTC ACC 270 226 A E Y A A P V K G 76 90 ATC TCA AGA GAT GAT TCA CAA AAC ACG CTG TAT CTG CAA ATG AAC 271 315 91 D S O N T Y 105 AGC CTG AAA ACC GAC GAC ACA GCC GTG TAT TAC TGT ACC ACA CAT 360 316 106 D D T A V Y 120 GTC TAC GGT GCC CCC CGG AAC TGG GGC CAG GGA TCC CTG GTC ACC 361 405 121 A P R N W G O G S 135 406 GTC TCC TCA GCC TCC ACC AAG GGC CCA AAG CTT GAA GAA GGT GAA 450 136 ASTKGP K L 451 TTT TCA GAA GCA CGC GTA CAG TCT GTG TTG ACT CAG CCG CCC TCA 495 151 EARVQ SVLTQ 165 496 GTG TCT GCG GCC CCA GGA CAG AAG GTC ACC ATC TCC TGC TCT GGA 540 166 P G 0 K T Ι 180 541 AGC AGC TCC AAC ATT GGA AAT AAT TAT GTC TCC TGG TAC GTT CAA 585 181 N I G N N y v S W 195 CTC CCA GGA ACA GCC CCC AAA CTC CTC ATT TAT GAC AAT AAT AAG 586 630 196 K r. 210 631 CGA TTC TCA GGA GTT CCT GAC CGA TTC TCT GGC TCC AAG TCT GGC 675 G v P D R G F S S 225 676 ACG TCA GCC ACC CTG GGC ATC ACC GGG CTC CAG ACT GGG GAC GAG 720 226 T L G I T G L 0 T 240 GCC GAT TAT TAC TGC GGA GCA TGG GAT GGC AGC CTG CGT GAA GCG 765 241 YYCGAWDGSLREA 255

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Continuation of Figure 26

766 256	GT.	A TT	C GG G	C GG/ G	A GGG	ACC T	AAG K	GT(ACC T	GT(CTA L	GGT G	GCG A	GCC A	GCA A	810 270
811 271	GT:	r ga	G CT	C GAC	GCG A	GCC A	GCG A	GAT D	TAC	AA.	GAC D	GAT D	GAC D	GAT D	AAA K	855 285
856 286	GA/	A TTO	G GG	A TC#	TCT	TCI	CGA R	ACC T	CCG	AGT S	GAC D	AAG K	CCT	GTA V	GCC A	900 300
901 301	CAT H	GT1	GT.	A GCA	AAC N	CCT	CAA Q	GCT A	GAG E	GGG G	CAG Q	CTC	CAG Q	TGG W	CTG L	945 315
946 316	AAC N	CGC R	C CGC	G GCC	AAT N	GCC	CTC	CTG L	GCC A	AAT N	GGC G	GTG V	GAG E	CTG L	AGA R	990 330
991	GAT	AAC	CAC	CTG	GTG	GTG	CCA	TCA	GAG	GGC	CTG	TAC	CTC	ATC	TAC	1035
331	D	N	Q		V	V	P	S	E	G	L	Y	L	I	Y	345
1036 346	TCC	CAG Q	GTC V	CTC	TTC F	AAG K	GGC G	CAA Q	GGC G	TGC	CCC	TCC S	ACC T	CAT H	GTG V	1080 360
1081	CTC	CTC	ACC	CAC	ACC	ATC	AGC	CGC	ATC	GCC	GTC	TCC	TAC	CAG	ACC	1125
361	L	L	T	H	T	I	S	R	I	A	V	S	Y	Q	T	375
1126	AAG	GTC	AAC	CTC	CTC	TCT	GCC	ATC	AAG	AGC	CCC	TGC	CAG	AGG	GAG	1170
376	K	V	N	L	L	S	A	I	K	S	P	C	Q	R	E	390
1171	ACC	CCA	GAG	GGG	GCT	GAG	GCC	AAG	CCC	TGG	TAT	GAG	CCC	ATC	TAT	1215
391	T	P	E	G	A	E	A	K		W	Y	E	P	I	Y	405
1216	CTG	GGA	GGG	GTC	TTC	CAG	CTG	GAG	AAG	GGT	GAC	CGA	CTC	AGC	GCT	1260
406	L	G	G	V	F	Q	L	E	K	G	D	R	L	8	A	420
1261	GAG	ATC	aat	CGG	CCC	GAC	TAT	CTC	GAC	TTT	GCC	gag	TCT	GGG	CAG	1305
421	E	I	N	R	P	D	Y	L	D	F	A	E	S	G	Q	435
1306	GTC	TAC	TTT	GGG	ATC	ATT	GCC	CTG	GGT	GGC	GGT	TCT	GGT	GGC	GGT	1350
436	V	Y	F	G	I	I	A	L	G	G	G	S	G	G	G	450
1351	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	TCA	TCT	TCT	CGA	ACC	CCG	1395
451	S	G	G	G	S	G	G	G	S	S	S	S	R	T	P	465
1396	AGT	GAC	AAG	CCT	gta	GCC	CAT	GTT	GTA	GCA	AAC	CCT	CAA	GCT	GAG	1440
466	S	D	K	P	V	A	H	V	V	A	N	P	Q	A	E	480
1441	GGG	CAG	CTC	CAG	TGG	CTG	AAC	CGC	CGG	GCC	AAT	GCC	CTC	CTG	GCC	1485
481	G	Q	L	Q	W	L	N	R	R	A	N	A	L	L	A	495
1486	AAT	GGC	GTG	GAG	CTG	AGA	GAT	AAC	CAG	CTG	gtg	GTG	CCA	TCA	GAG	1530
496	N	G	V	E	L	R	D	N	Q	L	V	V	P	S	E	510
1531	GGC	CTG	TAC	CTC	ATC	TAC	TCC	CAG	GTC	CTC	TTC	AAG	GGC	CAA	GGC	1575
511	G	L	Y	L	I	Y	S	Q	V	L	F	K	G	Q	G	525
1576 526	TGC C	CCC	TCC S	ACC	CAT H	GTG V	CTC	CTC .	ACC T	CAC H	ACC T	ATC I	AGC S	CGC R	ATC I	1620 540

Continuation of Figure 26

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1621 541	GC0 A	V CTC	S	Y	CAG Q	T	: AAG	GTC V	AAC N	L	CTC L	TCT	GCC A	ATC	AAG K	1665 555
1666	AGC	CCC	TGC	CAG	AGG	GAG	ACC	CCA	GAG	GGG	GCT	GAG	GCC	AAG	CCC	1710
556	S	P		Q	R	E	T	P	E	G	A	E	A	K	P	570
1711 571	TGG W	TAT Y	GAG E	P	ATC	TAT Y	CTG L	GGA G	GGG G	GTC V	TTC F	CAG Q	CTG L	GAG E	AAG K	1755 585
1756	GGT	GAC	CGA	CTC	AGC	GCT	GAG	ATC	AAT	CGG	CCC	GAC	TAT	CTC	GAC	1800
586	G	D	R	L	S	A	E	I	N	R		D	Y	L	D	600
1801	TTT	GCC	GAG	TCT	GGG	CAG	GTC	TAC	TTT	GGG	ATC	ATT	GCC	CTG	GGT	1845
601	F	A	E		G	Q	V	Y	F	G	I	I	A	L	G	615
1846	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGT	TCT	GGT	GGC	GGA	TCA	1890
616	G	G	S	G	G	G	S	G	G	G	S	G	G	G	S	630
1891 631	TCA S	TCT	TCT S	CGA R	ACC T	CCG P	AGT S	GAC D	AAG K	CCT	GTA V	GCC A	CAT H	GTT V	GTA V	1935 645
1936	GCA	AAC	CCT	CAA	GCT	GAG	GGG	CAG	CTC	CAG	TGG	CTG	AAC	CGC	CGG	1980
646	A	N		Q	A	E	G	Q	L	Q	W	L	N	R	R	660
1981	GCC	AAT	GCC	CTC	CTG	GCC	AAT	GGC	GTG	GAG	CTG	aga	GAT	AAC	CAG	2025
661	A	N	A	L	L	A	N	G	V	E	L	R	D	N	Q	675
2026	CTG	gtg	GTG	CCA	TCA	GAG	GGC	CTG	TAC	CTC	ATC	TAC	TCC	CAG	GTC	2070
676	L	V	V	P	S	E	G	L	Y	L	I	Y	S	Q	V	690
2071	CTC	TTC	AAG	GGC	CAA	GGC	TGC	CCC	TCC	ACC	CAT	gtg	CTC	CTC	ACC	2115
591	L	F	K	G	Q	G	C		S	T	H	V	L	L	T	705
2116	CAC	ACC	ATC	AGC	CGC	ATC	GCC	GTC	TCC	TAC	CAG	ACC	AAG	GTC	AAC	2160
706	H	T	I	S	R	I	A	V	S	Y	Q	T	K	V	N	720
2161	CTC	CTC	TCT	GCC	ATC	AAG	AGC	CCC	TGC	CAG	AGG	GAG	ACC	CCA	GAG	2205
221	L	L	S	A	I	K	S	P	C	Q	R	E	T	P	E	735
206 36	GGG G	GCT A	gag E	GCC A	AAG K	CCC	TGG W	TAT Y	GAG E	CCC	ATC I	TAT Y	CTG L	GGA G	GGG G	2250 750
251	GTC	TTC	CAG	CTG	GAG	AAG	GGT	GAC	CGA	CTC	AGC	GCT	GAG	ATC	AAT	2295
51	V	F	Q	L	E	K	G	D	R	L	S	A	E	I	N	765
296	CGG	CCC	GAC	TAT	CTC	GAC	TTT	GCC	GAG	TCT	GGG	CAG	GTC	TAC	TTT	2340
66	R		D	Y	L	D	F	A	E	S	G	Q	V	Y	F	780
341 81	GGG G	ATC I	ATT	GCC A	CTG L	TGA *										2358 785

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TNF Pharmacokinetics

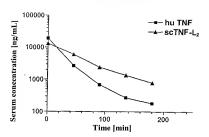


Figure 27

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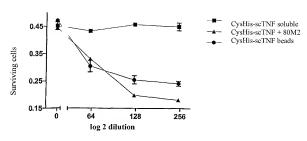


Figure 28